October 9, 1956

Dear Bernie:

Thank you for the cultures and letter of the 1st. As to the note, I am a little annoyed with SCIENCE, though it might be a better vehicle, and have already sent it to J. Bact. I hope they will let me have it back for corrections so I can consider applying some of your suggestions.

I also got in touch with Park, and found he and Strominger have reached the same conclusions from the chemical end, namely by the identification of the same residues previously isolated as UDP- conjugates as cell-wall constituents. I am trying to make some arrangement with Park for concurrent publication, as the morphology and chemistry (though on different organisms) fit together so nicely.

As to 173-25, WE are having a great deal of trouble with reversion. A direct plating from your slant(s) on A (= D[c]) agar + 20 mcg/ml lysine plus 5 mcg/ml DAP gives a profusion of prototrophs (i.e. by comparison with unsuppl. medium) and a scattering of minute colonies between them. The latter are presumably the auxotroph. New prototrophs are arising from these with continued incubation. Apparently, we are not using enough DAP, and are fooling with this now so we can reisolate and maintain the culture. I didn't (and don't) know whether penassay has any DAP or not? Do you? I suspected not, but how did you manage to isolate the culture in the first place? Our first tests were done in parallel with the slant received and a penassay passage.

By forthcoming ms., I assume you mean an account of your current tests with DAP/sucrose. I am glad you are doing this, as it may relieve some potential embarrassment. My main interest is to tie in with the morphological angles on the penicillin-protoplasts. I can't say anything more definite till we have a reliable inoculum. (If you rexperience suggests another difficulty than boosting the DAP, I'd appreciate hearing about it. I am giving these details in hopes of exposing an interesting or useful discrepancy.) What the mixed cultures suggest (ie. from ignoring the heavy overgrowth) is:

1. Lysine stimulatesgrowth slightly. 2. [illegible] In absence of DAP, the mutant cells swell up and lyse. 3. If lysine is withheld (as well as DAP) the cells do not swell. 4. If sucrose is added, the cells do not swell. There was no indication that sucrose would allow either growth (in absence of DAP) or the development of protoplasts; it simply seemed to prevent the swelling and lysis. These (presumably) osmotically fragile rods may be analogous to the lysozyme-treated rods that Norton found, which require further treatment (e.g. high pH or versene) to transform them into protoplasts. This is rather complicated, so I'm repeating the observations on the attached table. They are not too reliable, as the inoculum was grossly impure.

Inoculum: Suspension of Davis' 173-25 (original slant) in water, streaked. All in minimal agar. Supplements: lysine at 20 mcg/ml; DAP 5; sucrose: 10%. Prototroph overgrowth heavy, but tried to ignore it.

| Suppl. | No sucrose | Sucrose |
|---|---|---|
| — | scattered rods | scattered rods |
| lysine | debris & spherical ghosts* | scattered rods [no protoplasts] |
| DAP | colonies | colonies |
| DAP+lysine ** | colonies | colonies |

*These are not intact protoplasts. They resemble the ghosts obtained when pc-protoplasts are lysed.

** growth sl. heavier than s/ DAP.
P.S. Have you looked at Toennies' paper?

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I hope these are consistent with your findings or, if not, that they suggest something useful.

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Gordon Allen answered my comments about his ms. He feels that Calvin has anticipated him completely, and that he [GA] has lost his chance for glory. I'm worried about him: the intensity of his preoccupation is certainly not healthy. It'a a pity the scientific community doesn't do more to protect itself from this competitive ratrace for spurious (cf. Shakespeare) goals, instead of encouraging it with prizes and medals etc. Most of us are more or less bitten by this disease, but I think the open competitions are what accentuate it. It certainly doesn't make for a calm intellectual atmosphere, but I may be wrong in thinking there'd be enough other motivations if these were sublimated.

I'll let you know how we make out further; don't rush to reply unless you can see where we're off the track

Yours,

Joshua Lederberg

Oct. 9: P.S.

The DAP level is the problem: we had to go to 50 mc/ml to get growth of the mutant which would compete effectively with reversions. We now have the means of making decent inocula. The DAP is the salt you provided, expressed as DAP. We've set up some of your lysineless culture, hopefully, as further source.

I may have to revise one remark above: in one further plating +lys + sucrose -dap, there were mostly distorted rods, ~~assembling~~ a partly reverted protoplast suspension, as ~~if~~ if the cells could make some sort of a wall without dap, but not a proper one. I don't know why the appearances differ from trial to trial.

Yours

JL